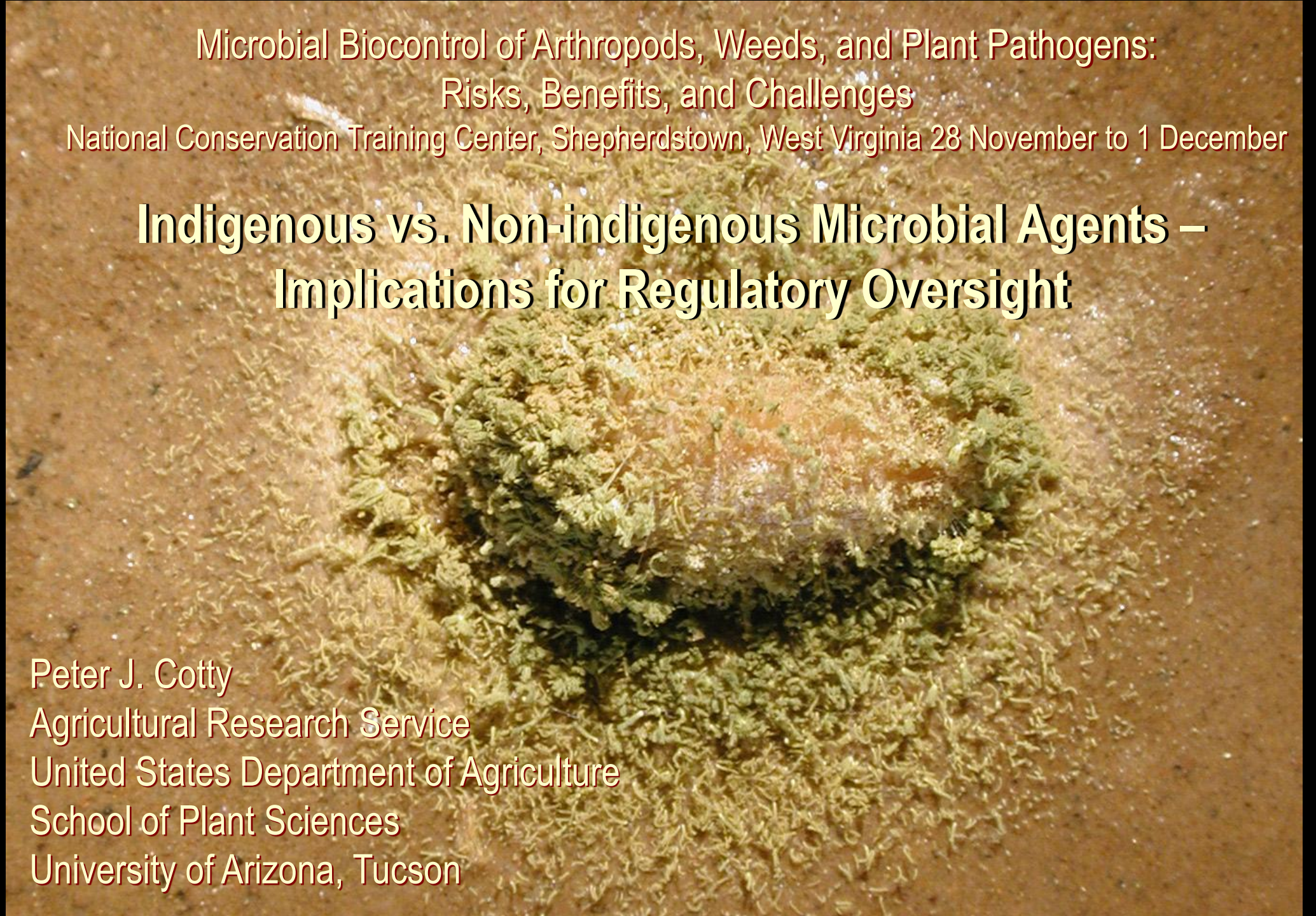
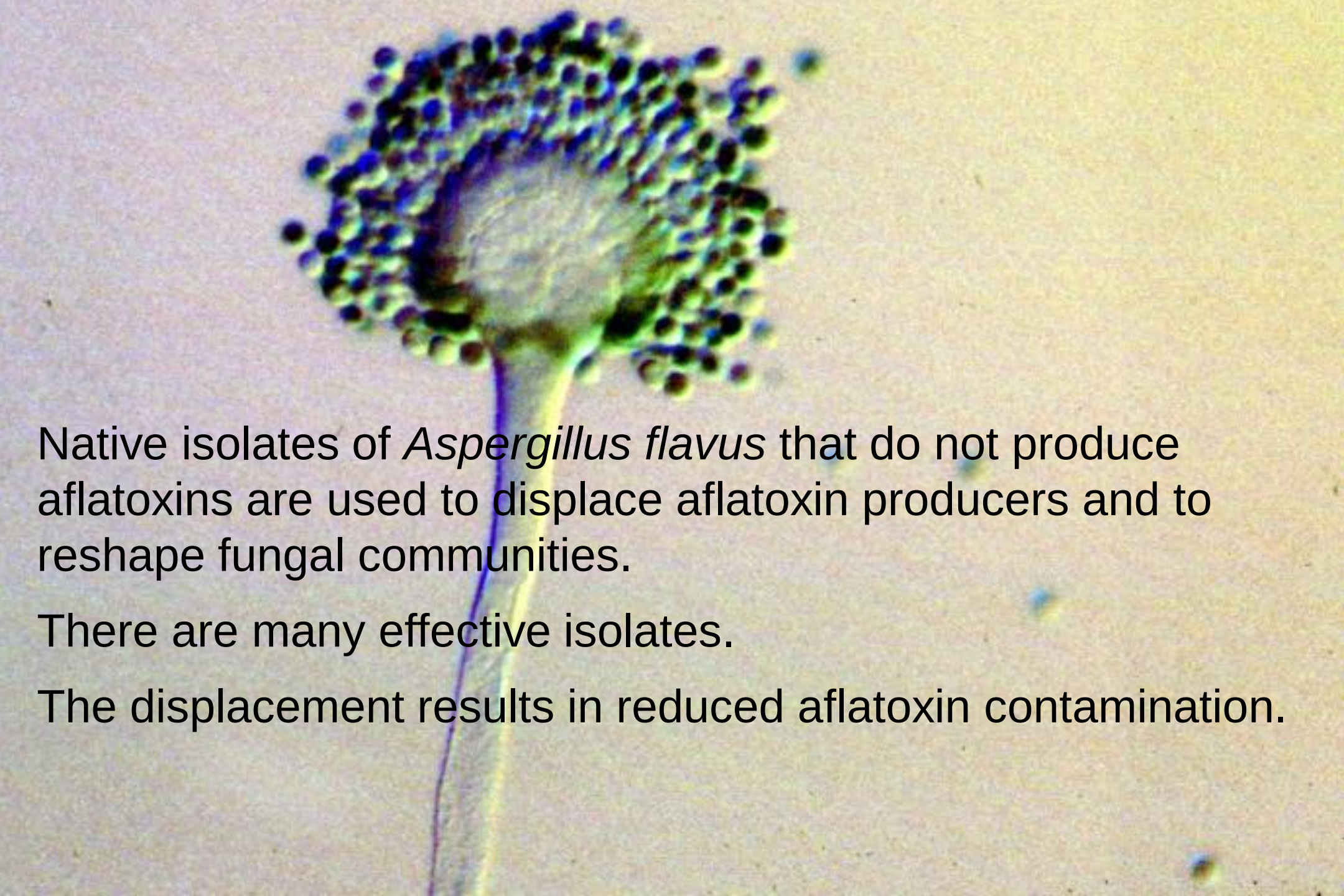
Microbial Biocontrol of Arthropods, Weeds, and Plant Pathogens:
Risks, Benefits, and Challenges
National Conservation Training Center, Shepherdstown, West Virginia 28 November to 1 December

Indigenous vs. Non-indigenous Microbial Agents – Implications for Regulatory Oversight



Peter J. Cotty
Agricultural Research Service
United States Department of Agriculture
School of Plant Sciences
University of Arizona, Tucson

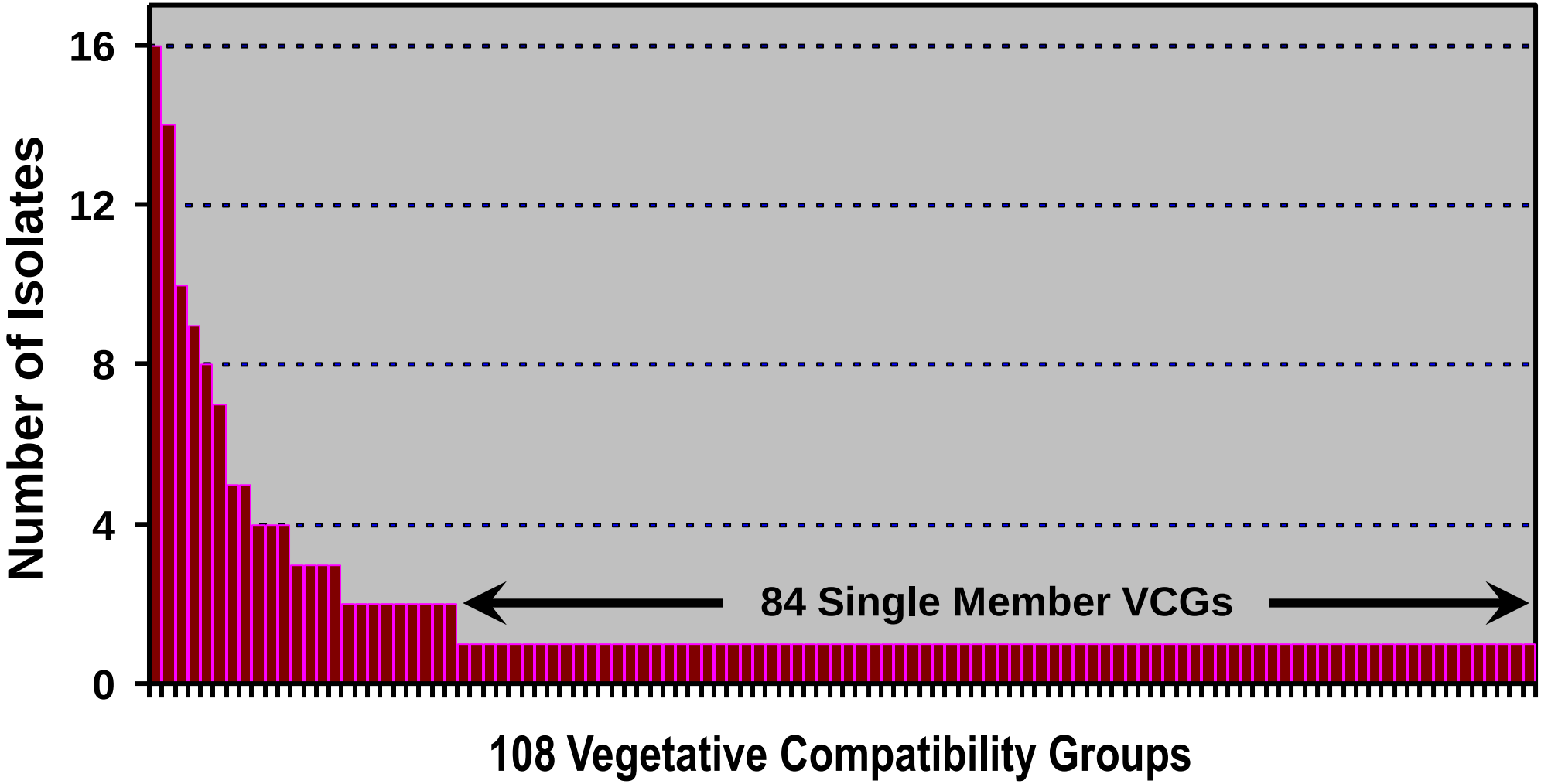


Native isolates of *Aspergillus flavus* that do not produce aflatoxins are used to displace aflatoxin producers and to reshape fungal communities.

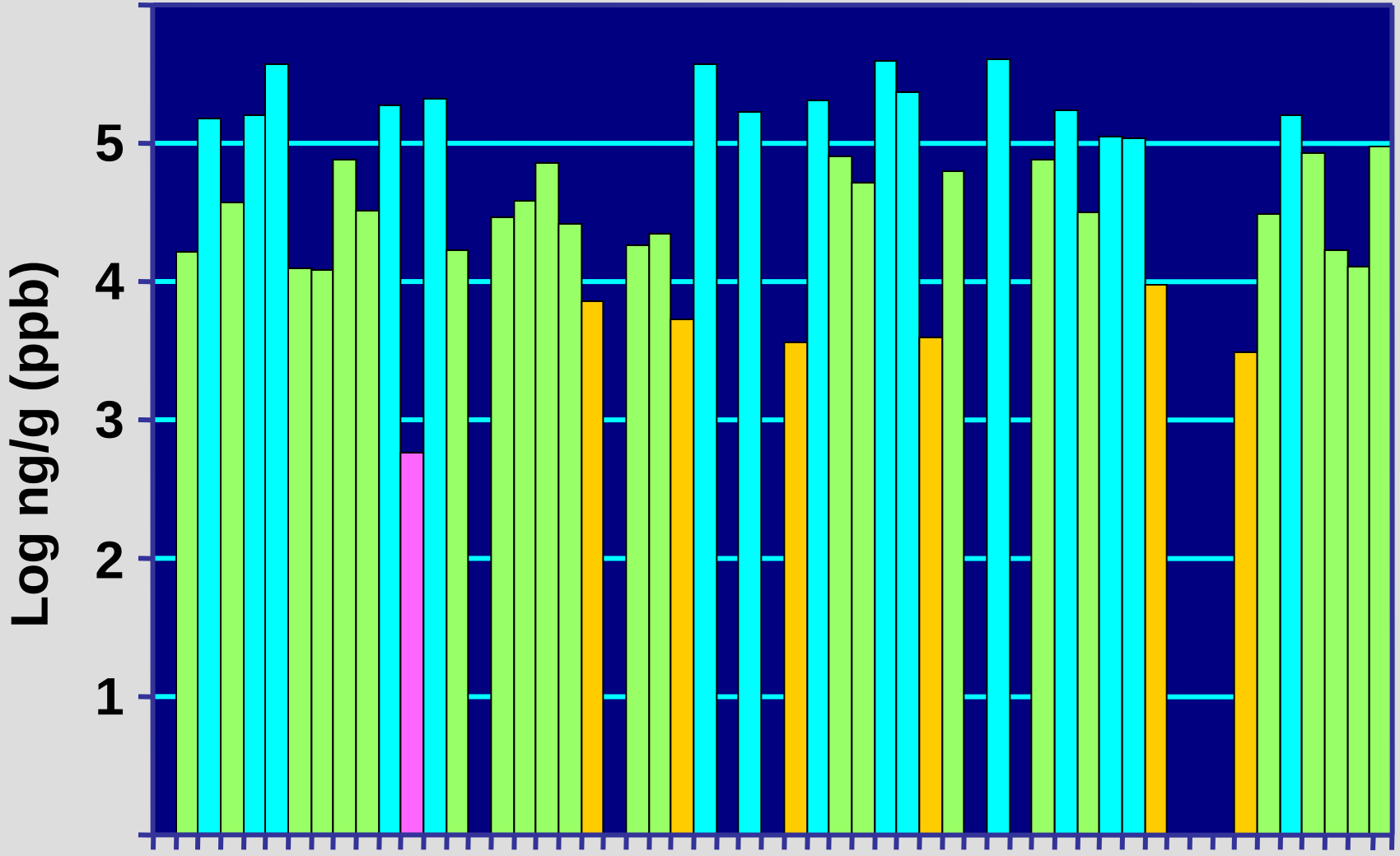
There are many effective isolates.

The displacement results in reduced aflatoxin contamination.

Frequencies of Vegetative Compatibility Groups among 200 L Strain Isolates of *Aspergillus flavus* isolated from Cotton in South Texas During 1999



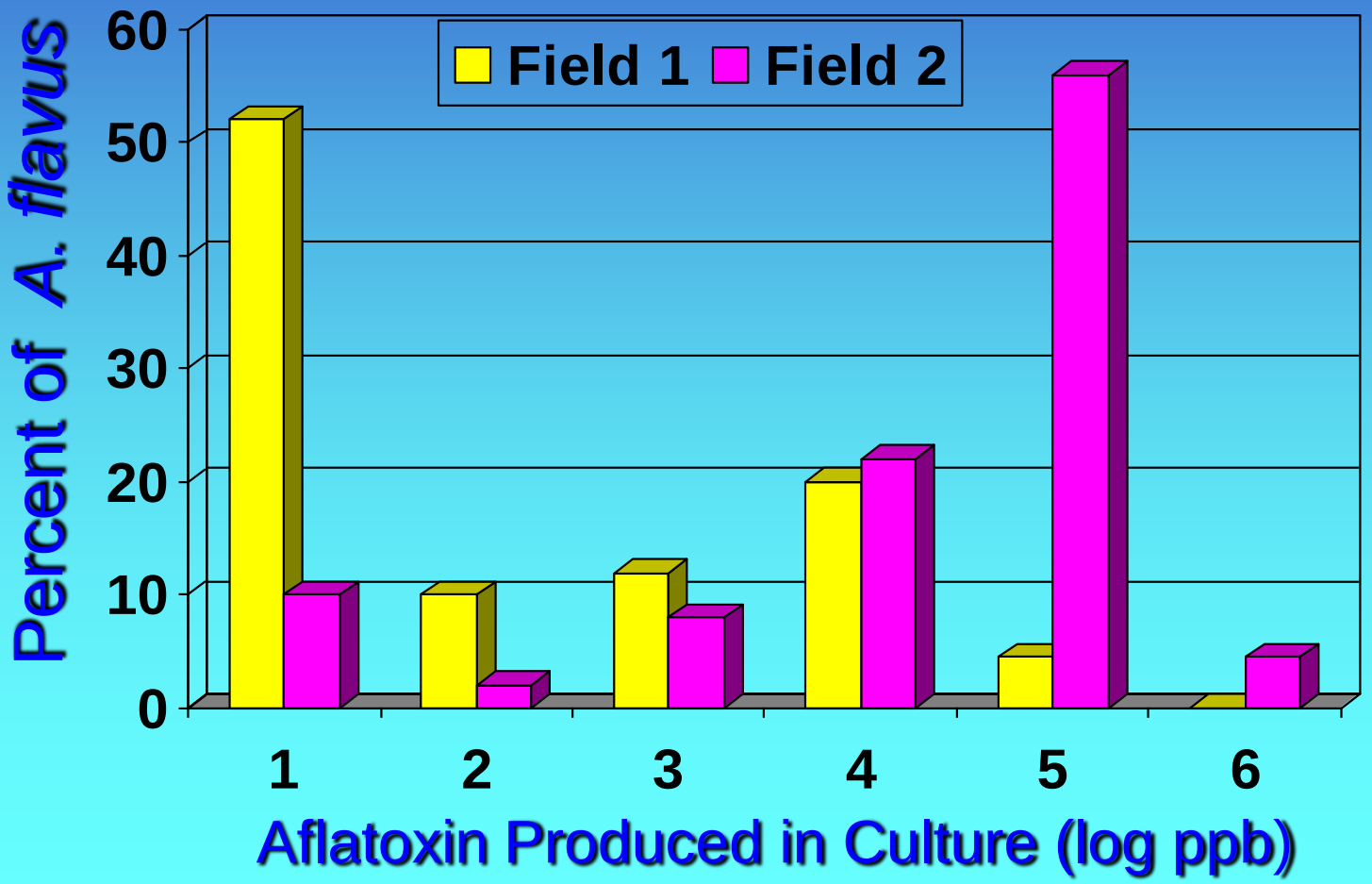
Aflatoxin Production by Fungal Isolates in Liquid Fermentation



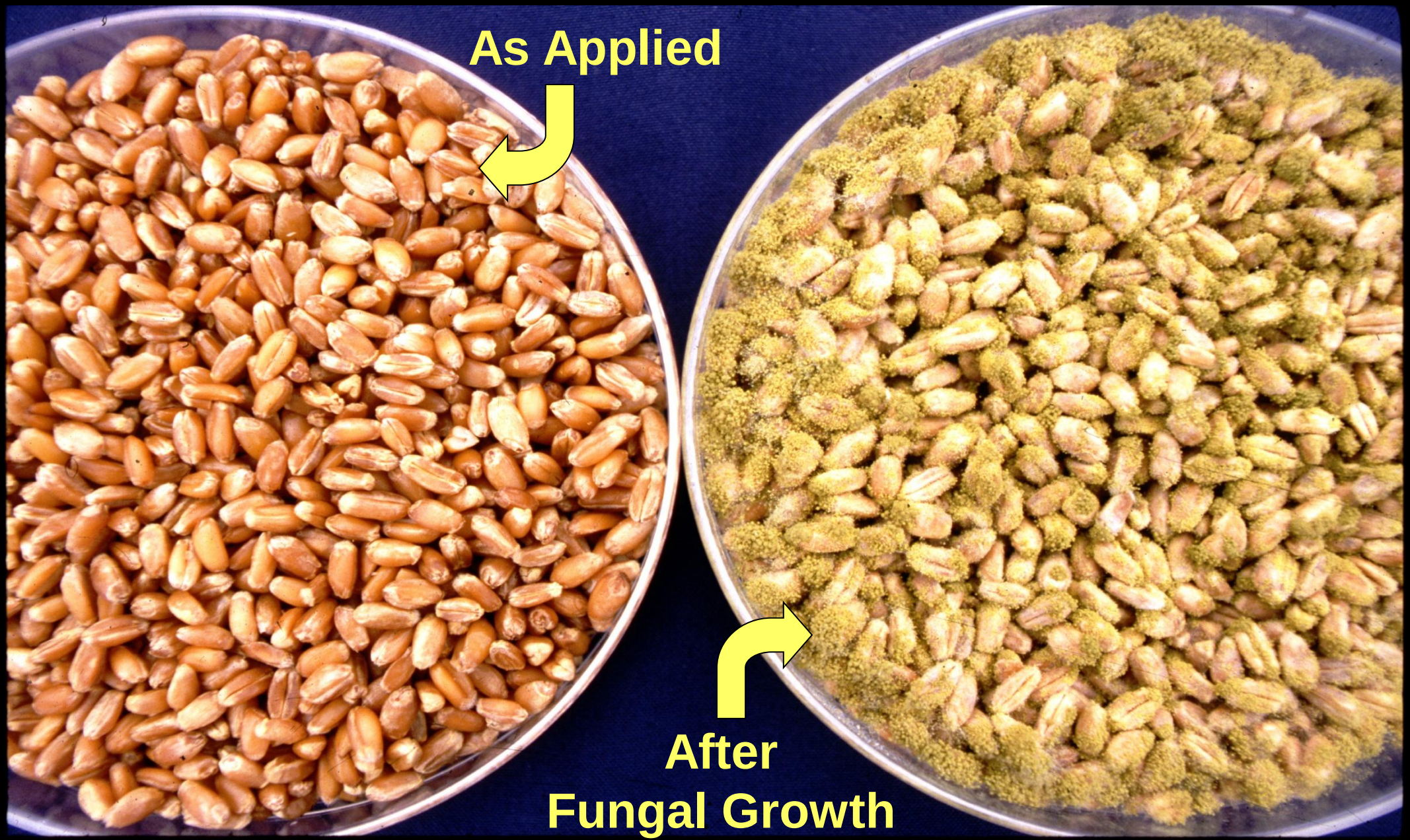
Fungi Vary Across Areas in Aflatoxin-Producing Ability

The average aflatoxin-producing potential of fungi on a farm influences the vulnerability of crops grow on that farm to aflatoxin contamination

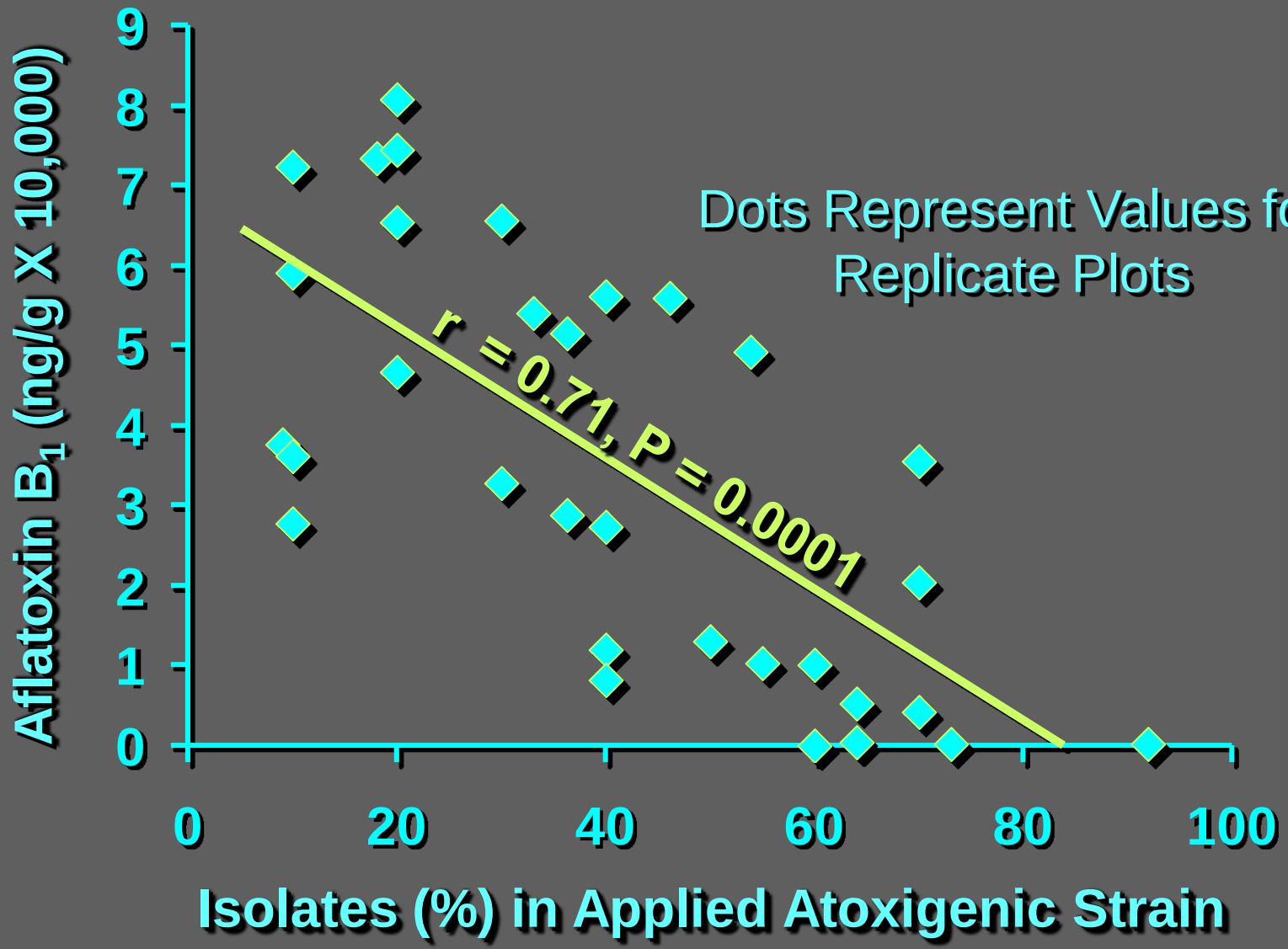
Aflatoxin Production by *A. flavus* from Two Fields



Aflatoxin-Producing Potential
Field 1 = Low, 3,400 ppb
Field 2 = High, 54,000 ppb


As Applied
After
Fungal Growth

Crop Aflatoxin Content Decreases as Incidence of the Applied Strain Increases



Influence of Field Application of Atoxigenic *A. flavus* on Aflatoxin, Infection, and the Total Amount of *A. flavus*

	Aflatoxin B_1 (mg kg^{-1})	Infection (%)	A. flavus on crop (propagules/g)	Applied strain (%)
Treated	0.3 b	1.03 a	23,949 a	100 a
Control	81.8 a	0.85 a	28,949 a	7 b

Values followed by a common letter do not differ significantly.

Commercial Maize Test: North Central Texas 2008

Area	Samples (#)	AF36 (%)	Aflatoxin (ppb)	
			Mean	Range
Grayson North	17	96 a	12 a	0 to 48
Grayson South	16	98 a	15 a	0 to 38
Grayson Control	8	24 b	230 b	5 to 530

Means in the same column with different letters are significantly different by Tukey's HSD test, $P < 0.001$.

Indigenous: Why a Preference?

Efficacy
Adapted to the target cropping system

Environmental Safety
The species is very broadly adapted, difficult to predict all influences of introducing an exotic

Competitiveness in Endemic Niches
Long-term influences

Acceptance
Farmer preference

Ownership
Owned by nation of origin

Administrative
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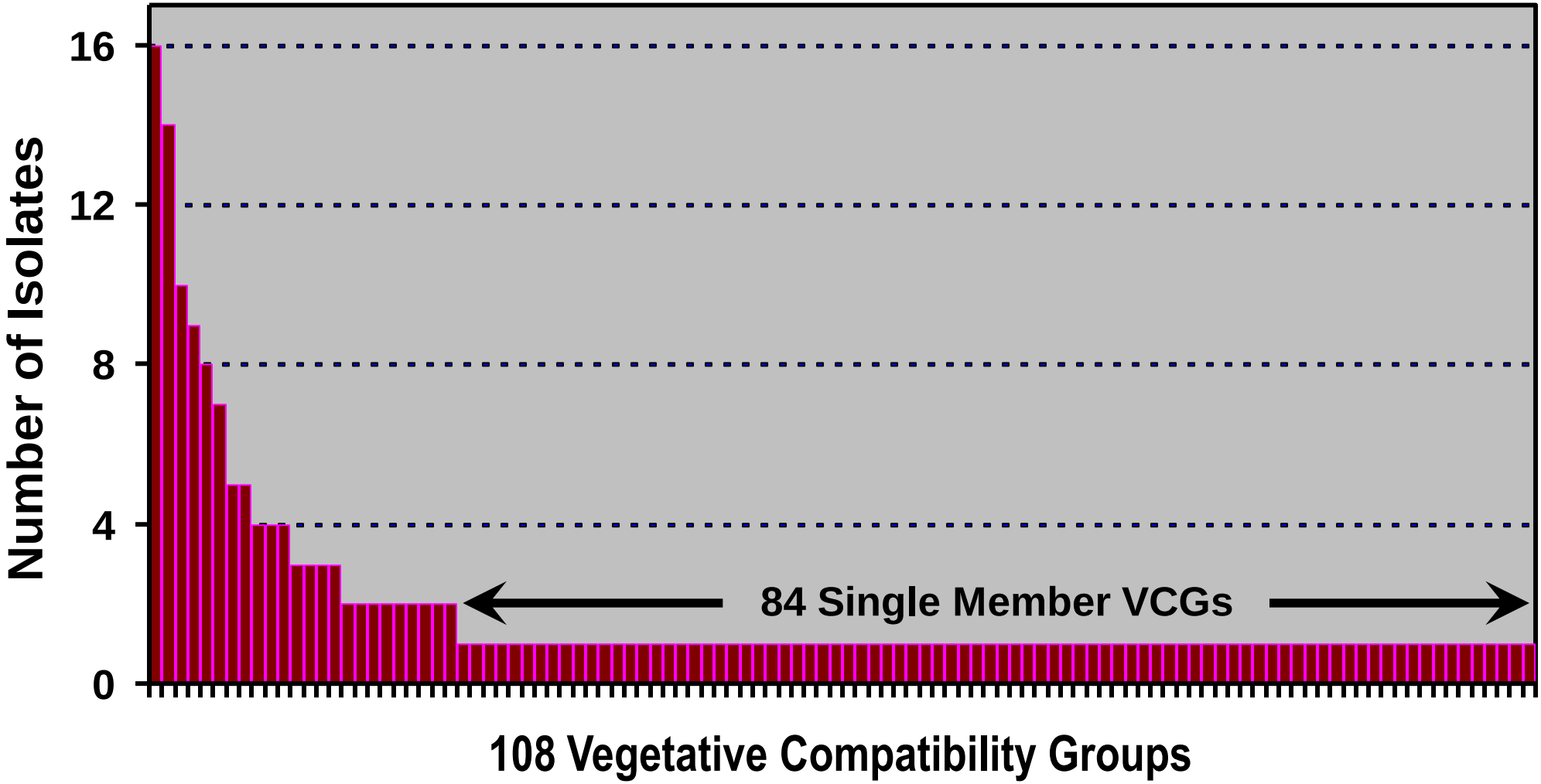
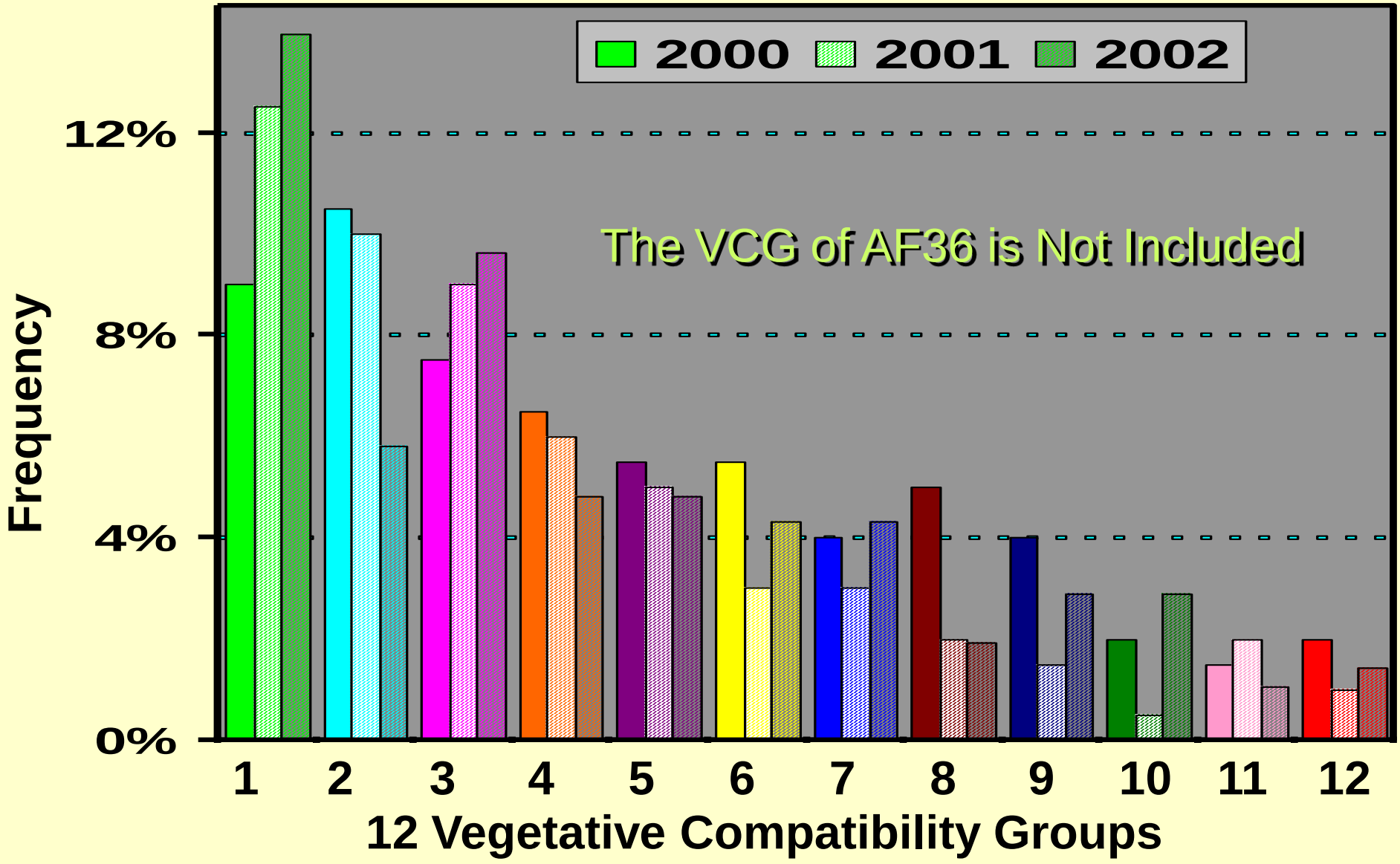


Frequencies of the 12 Most Common Vegetative Compatibility Groups on Cotton In Arizona from 2000 to 2002



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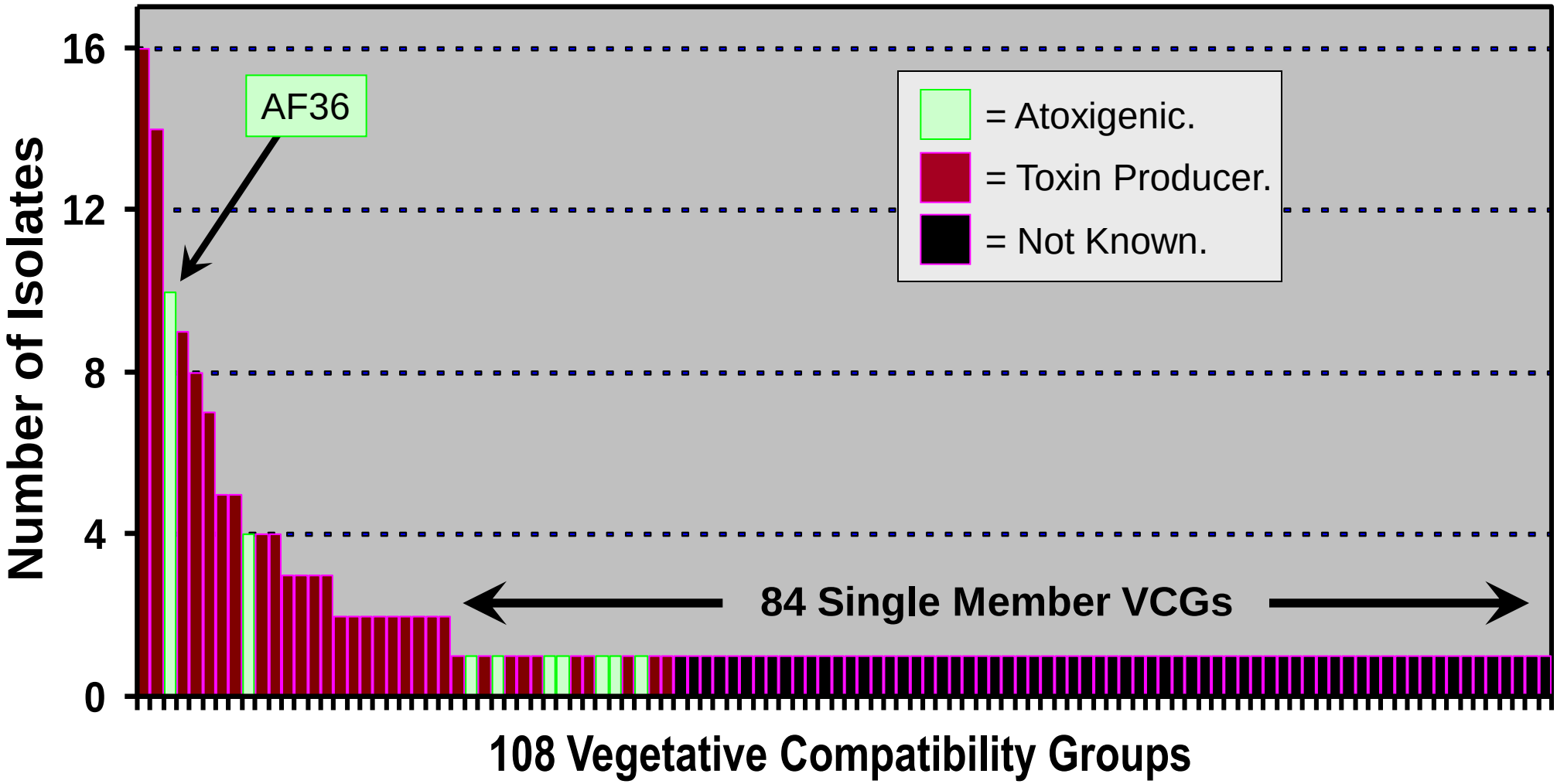

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In Native Desert Areas of the Sonoran Desert both Aflatoxins and *Aspergillus flavus* are Very Common

Boyd & Cotty, 2001.
Phytopathology 91:913-919.

A. flavus and aflatoxins in pods of four common legumes in the Sonoran Desert

Legume Pod	Samples (#)	log CFU	Aflatoxin		
			Mean (ppb)	Max (ppb)	Positive (%)
Mesquite (immature)	5	1.22	0	0	0%
Mesquite (on tree)	39	5.50	36	352	23%
Mesquite (from ground)	15	6.39	178	2,672	33%
Acacia*	5	3.68	0	0	0%
Palo Verde	18	3.03	18	318	6%
Ironwood*	12	3.53	16	154	33%

*Ironwood & Acacia only dehisced pericarps from ground.

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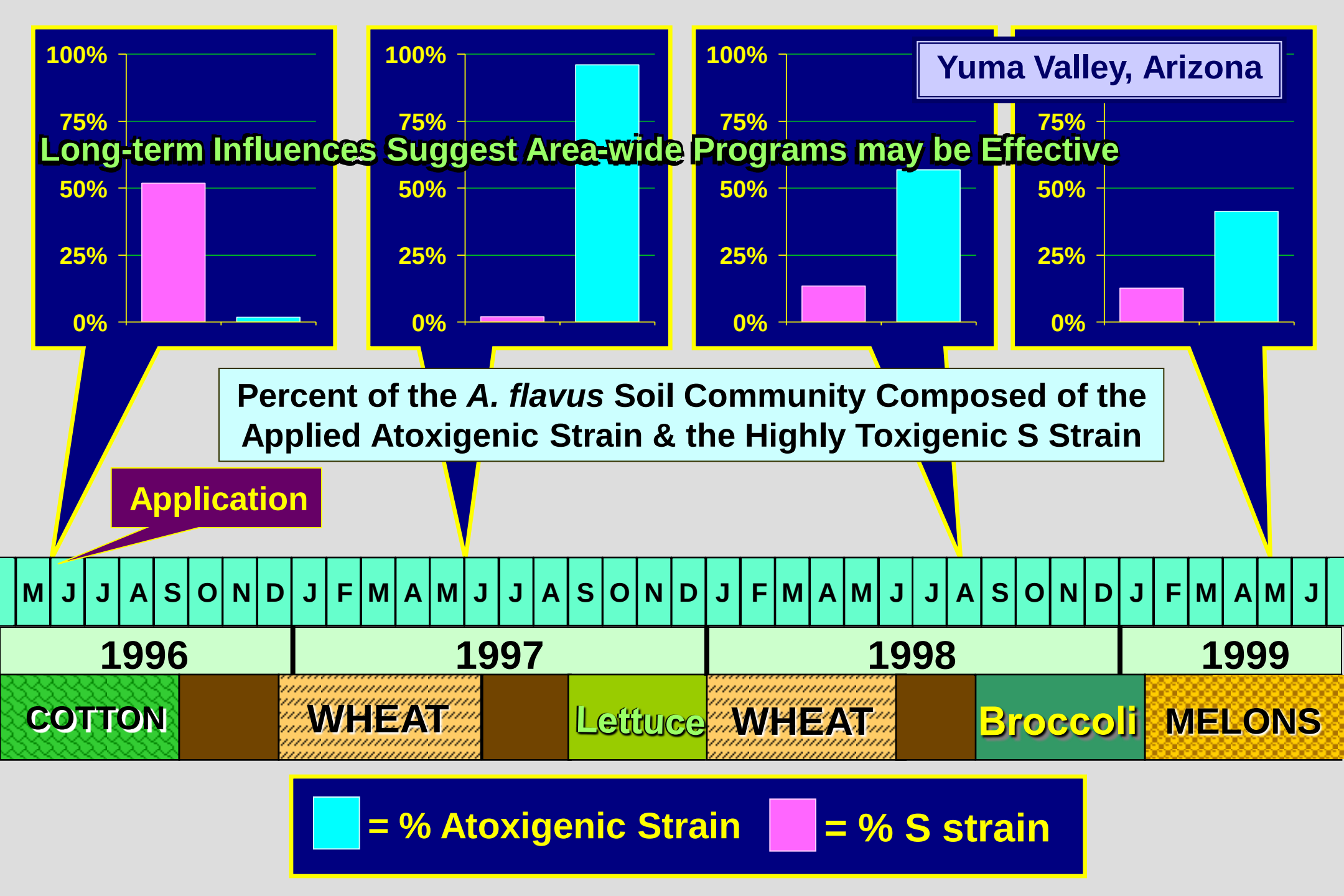
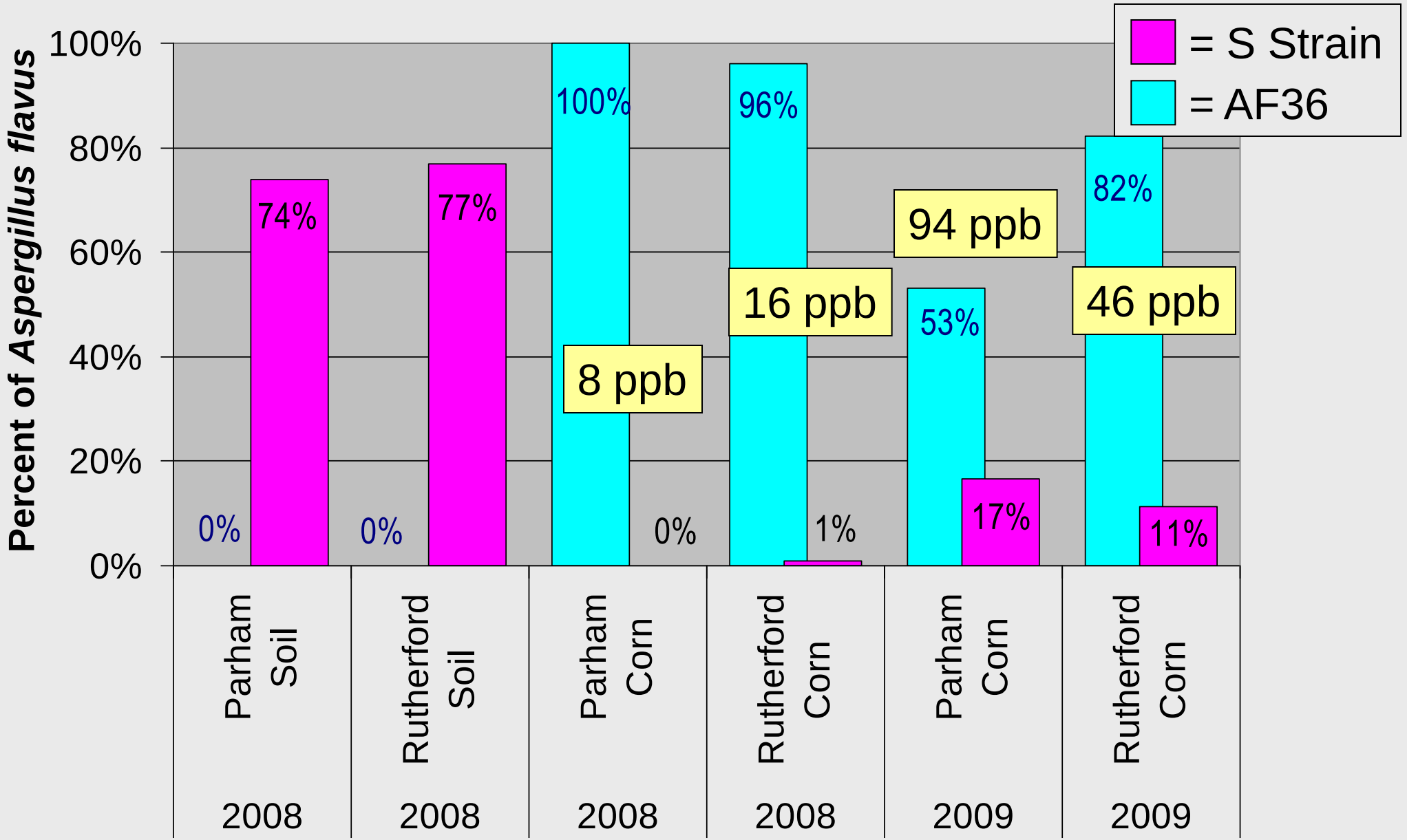
Composition of *Aspergillus flavus* Communities in Soil of Treated and Nearby Fields in May 1996 Prior to Application of AF36 and in May 1997 One Year After Application

Field type	Fields (#)	AF36 (% *A. flavus*)		S strain (% *A. flavus*)		*A. flavus* (CFU/gram)	
		1996	1997	1996	1997	1996	1997
Treated	3	4% ab	85% a	52% a	4% d	582 a	365 a
Adjacent	4	2% b	48% b	41% a	18% c	411 a	157 a
Diagonal	4	2% b	16% c	52% a	33% b	61 a	100 a
Other	4	9% a	9% c	43% a	50% a	109 a	98 a

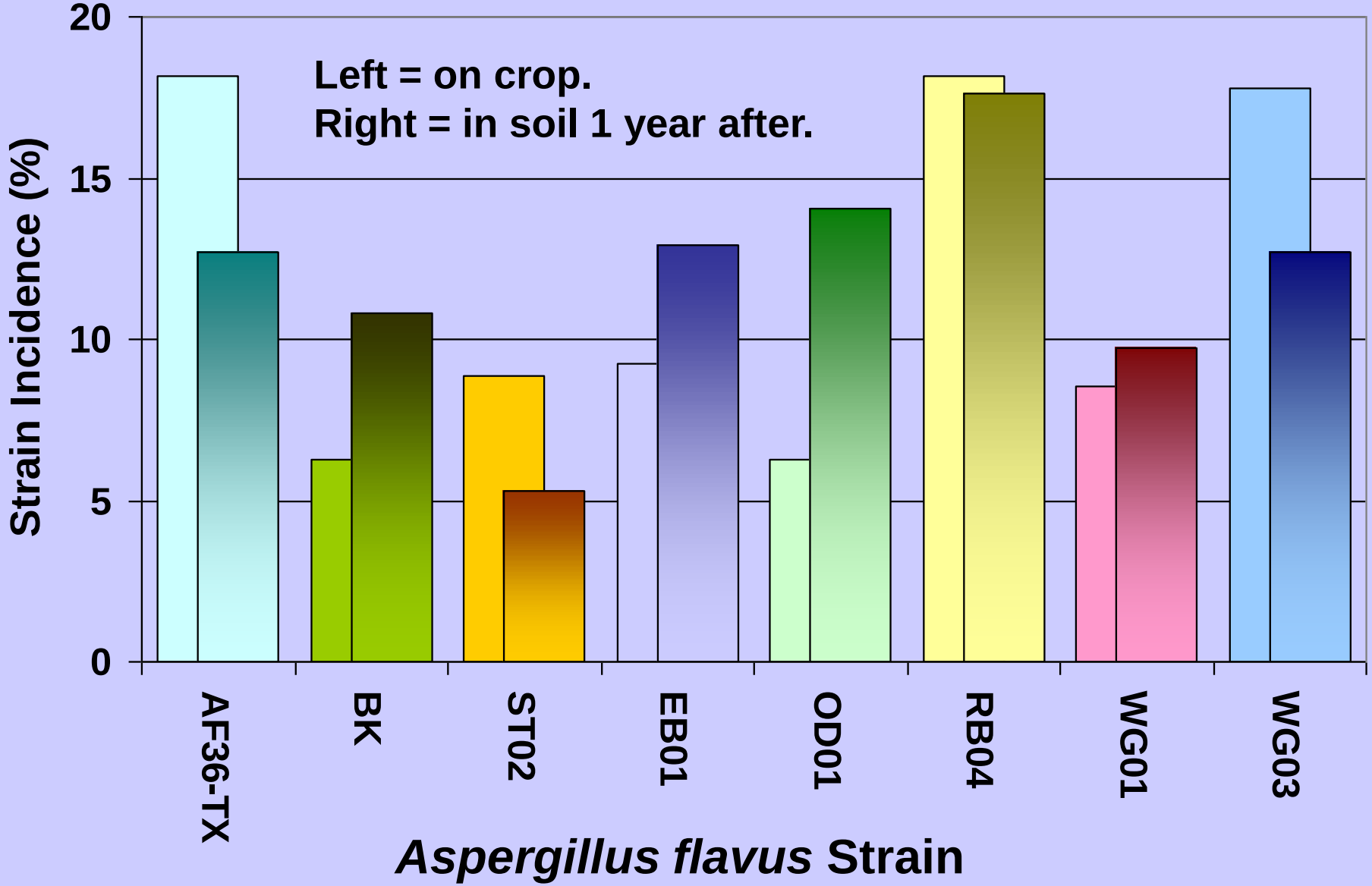
Other	Adjacent	Treated	Adjacent	Other	Other
Other	Diagonal	Adjacent	Diagonal	Other	Other


Yuma Valley, Arizona
Long-term Influences Suggest Area-wide Programs may be Effective
100%
75%
50%
25%
0%
Percent of the A. flavus Soil Community Composed of the Applied Atoxigenic Strain & the Highly Toxigenic S Strain
Application
M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J
1996
1997
1998
1999
COTTON
WHEAT
Lettuce
WHEAT
Broccoli
MELONS
= % Atoxigenic Strain
= % S strain

Grayson County: Carry Over to the Second Year Crop



Incidence of Eight *A. flavus* Strains on Treated Crops and in Soil 1 Year After Application - Average of 3 Trials



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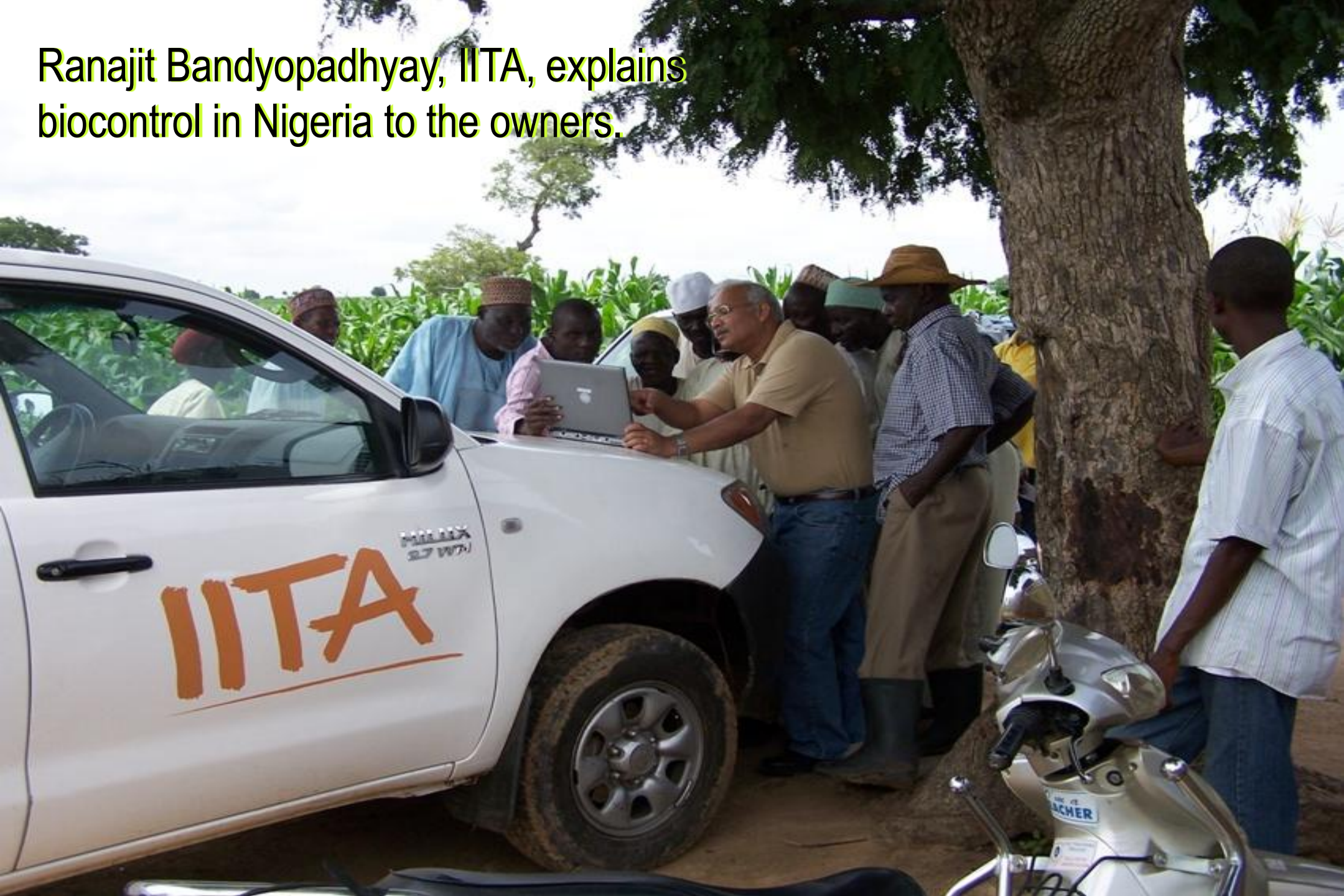

Ranajit Bandyopadhyay, IITA, explains biocontrol in Nigeria to the owners.

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NAFDAC officials inspecting maize fields treated with AflaSafe

The Initial Atoxigenic Strain Pesticide Registration
A Public Sector Effort

Biopesticide Registration of *Aspergillus flavus* AF36 Milestones

1993 - First Meeting with EPA

1995 - IR-4 Biopesticide Program Joined the Effort

1996 - First EUP Granted Allowed Treatment of 1120 acres over 3 year period (1996 through 1998).

1999 - EUP expanded to include 20,000 acres per year.

2002 - EUP expanded to include 20,000 acres in Arizona and 2,000 acres in Texas.

2003 - Section 3 registration granted allowing treatment of unlimited acreage in Arizona & Texas.

2007 - EUP to Treat 3,000 acres of pistachios in California. Approved in 2008 by CDPR.

2008 - EUP to treat 6,000 acres of corn. Pistachios expanded to include 1,000 acres in Arizona.

Crops are infected by complex communities of diverse fungi

We can influence aflatoxin-producing ability of fungal communities resident in production areas through crop rotations, agronomic practice, and by applying atoxigenic strains

There are many atoxigenic strains

Select strains best adapted to rotations, ecosystems, & climates

Atoxigenics are Already Present on the Crop

Just increasing the frequency of endemic strains & natural interference with contamination

Treatments May have Long-Term Influence & Cumulative Benefits

More than One Crop May Benefit From the Same Strain

Atoxigenic Strains can be Applied Without Increasing Infection

and without increasing the overall quantity of *A. flavus* on the crop & throughout the environment